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Intra- and intersexual interactions shape microbial community dynamics in the rhizosphere of *Populus cathayana* females and males exposed to excess Zn

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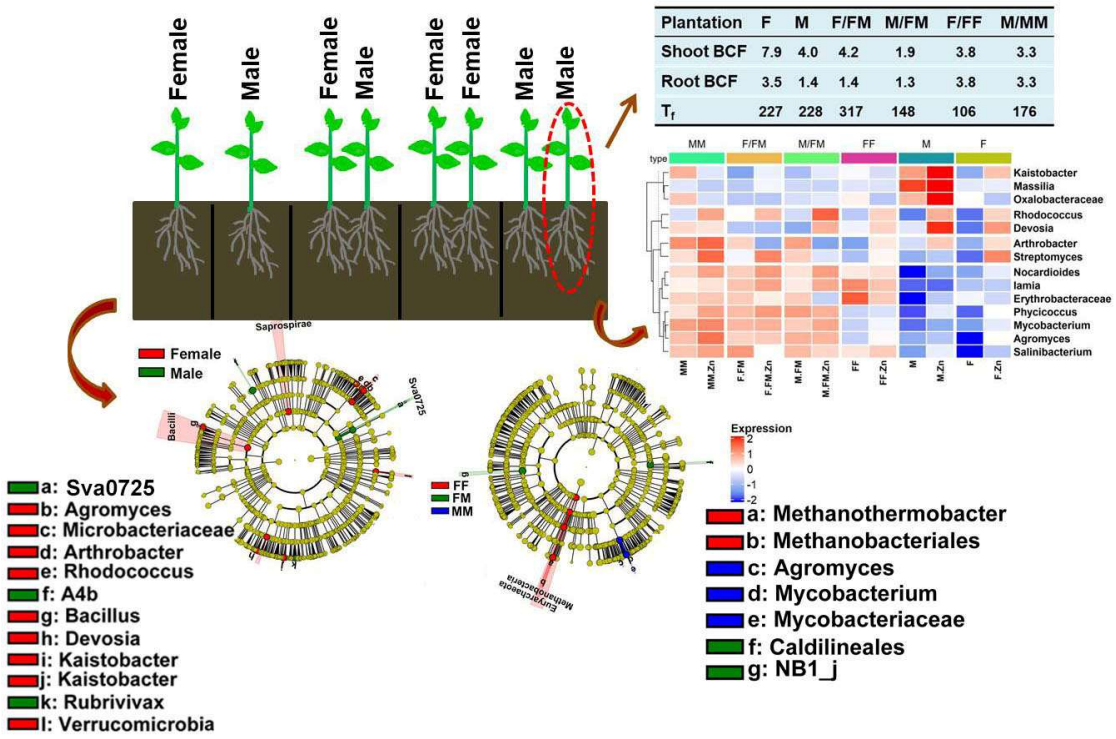
Abstract Although increasing attention has been paid to rhizospheric bacteria in plants in relation to the bioavailability and tolerance of heavy metals, interactive effects between sex and microbiological processes on phytoextraction have been overlooked, especially in dioecious plants. In this study, we intended to investigate the responses of rhizospheric bacterial communities of *Populus cathayana* Rehder to excess Zn under different planting patterns. The results suggested that intersexual and intrasexual interactions strongly affect plant growth and Zn extraction in both sexes, as well as rhizosphere-associated bacterial community structures. Females had a higher capacity of Zn accumulation and translocation than males under all planting patterns. Males had lower Zn accumulation and translocation under intersexual than under intrasexual interaction; the contrary was true for females. Females harbored abundant Streptomyces and Nocardioides in their rhizosphere, similarly to males under intersexual interaction, but differed from single-sex males under excess Zn. Conversely, intersexual interaction increased the abundance of key taxa Actinomycetales and Betaproteobacteria in both sexes exposed to excess Zn. Males improved the female rhizospheric microenvironment by increasing the abundance of some key tolerance taxa of Chloroflexi, Proteobacteria and Actinobacteria in both sexes under excess Zn in intersexual interaction. This was associated with metal activation and bioavailability. Females harbored abundant Methanothermobacter, while males had abundant Mycobacterium in their rhizosphere under intrasexual interaction. These results indicated that the sex of neighboring plants affected sexual differences in the choice of specific bacterial colonizations for phytoextraction and tolerance to Zn-contaminated

soils, which might regulate the spatial segregation and phytoremediation potential of *P. cathayana* females and males under heavy metal contaminated soils.

Key words: sexual interaction; excess Zn; bacterial abundance; bacterial community; rhizosphere.

Graphical abstract



1. Introduction

Males and females of dioecious plant species differ in their sexual and vegetative traits, including physiology, phenology, and allocation to reproduction and defense (Chen et al., 2014; Tonnabel et al., 2017; Xia et al., 2020). Sex-related differences in these traits are probably associated with differences in reproductive costs. Females allocate more resources to reproduction than males, since females produce not only flowers but also fruits and seeds (Juvany and Munné-Bosch, 2015). The maintenance of reproductive vigor usually limits vegetative growth and defense investment. Thus, when resources are limited, reproduction directly competes with the other two processes, leading to reduced stress tolerance (Mercer and Eppley, 2010; Tonnabel et al., 2017). It has been shown that females generally allocate more resources to reproduction and males often increase investment into defensive responses under natural conditions (Juvany and Munné-Bosch, 2015). Our previous studies have suggested that males usually exhibit a higher tolerance to stressful environments when compared with females (Zhao et al., 2012; Chen et al., 2014; Li et al., 2016; Zhang et al., 2019).

Zinc (Zn) is an essential micronutrient with a series of critical roles in living organisms; yet, Zn can be toxic at elevated concentrations (Broadley et al., 2007; Lu et al., 2013). It has been suggested that Zn toxicity symptoms usually appear when the leaf Zn content is > 30 $mg \cdot kg^{-1}$ of leaf dry weight, although the toxicity threshold may be highly variable, even in the same species (Ayangbenro and Babalola, 2017). Excess Zn affects

plant growth and development, as well as threatens the health of animals due to excessive amounts of Zn ending up into the food chain (Duruibe et al., 2007; Carruthers, 2016). The threat of excess Zn to the health of living organisms in the environment is exacerbated by its persistent nature (Ayangbenro and Babalola, 2017). Therefore, it is critical to remediate Zn-polluted soils. Several fast-growing trees, including poplars, are regarded as promising candidates for phytoremediation of heavy metal-polluted soils due to their high photoabsorption capacity and biomass, as well as reduced impact on the food chain (Chen et al., 2011; He et al., 2015; Li et al., 2017).

The migration and transformation of heavy metals in rhizospheric soils largely depend on the physical and chemical properties of soil (Rasmussen et al., 2000; Weng et al., 2014), plant types (Hou et al., 2017), rhizospheric microorganisms (Ayangbenro and Babalola, 2017) and exudates (Tao et al., 2020). Among these, rhizospheric microbial interactions with heavy metals play a critical role in heavy metal uptake and tolerance (Rajkumar et al., 2012). Microorganisms have developed variable mechanisms for maintaining heavy metal homeostasis and resistance, including biomineralization, bioaccumulation, biotransformation and biosorption (Navarro-Noya et al., 2010; Mishra et al., 2017). Heavy metals have been shown to suppress microorganisms' growth, and alter their cell morphology and biochemical characteristics, which reduces microbial biomass and diversity (Ayangbenro and Babalola, 2017). Still, microorganisms have evolved diverse defense mechanisms that help them cope with the toxic effect of heavy metals. Burd et al. (2000) have suggested that a metal-resistant

bacterium *Kluyvera ascorbate SUD 165* alleviates growth inhibition caused by a high level of nickel in *Brassica campestris*. Hou et al. (2017) have also reported that the rhizospheric bacterium *Streptomyces* probably promotes Cd accumulation in the hyperaccumulator plant *Sedum alfredii*. Therefore, the coexistence of plant and microbial systems not only facilitates survival in heavy-metal contaminated soils, but also the removal of heavy metal.

Interspecific and/or intraspecific neighbor interactions of plants play critical roles in determining the structure and function of biological communities (Chen et al., 2014). Intra- and interspecific competitive capacities have important implications for phytoremediation in metal-hyperaccumulating species (Arthur et al., 2005; Pilon-Smits and Freeman, 2006). Interactions have been shown to alter plants' responses to nickel and Zn (Koelbener et al., 2008). Zhao et al. (2017) have found that interspecific interactions can enhance antioxidant enzyme activities that increase survival and fitness in plants exposed to multiple metal stresses. Chen et al. (2016) have also shown that resource competition between consexual and heterosexual neighboring plants affect Cd allocation, biomass partitioning and carbon-nutrient balance in poplars exposed to Cd stress. Interactions not only alter plants' morphological and physiological responses, such as nutrient uptake, heavy metal availability and root exudate secretion, but also affect microbial communities in soil (Guo et al., 2019). So far, the effects of intersexual and intrasexual interactions on belowground microbial communities have been largely overlooked, especially under heavy metal stress.

Populus cathayana Rehder is widely distributed in China and is regarded as a major forestry species with a high commercial and ecological value. *P. cathayana* is a common dioecious plant that displays sexual dimorphism (Chen et al., 2014; Zhang et al., 2019; Xia et al., 2020). Recently, some studies have suggested that poplar species have different tolerance mechanisms and phytoremediation potential to heavy metals, mainly based on genetic differences (Chen et al., 2017; Bi et al., 2020; Liu et al., 2020b). In addition, *Populus* females and males display sexually different physiological responses to heavy metal stress, and males usually show a stronger tolerance when compared to females (Chen et al., 2016, 2017; Liu et al., 2020a, b). Sex-specific responses to abiotic factors are affected by inter- and/or intraspecific interactions (Chen et al., 2016, 2017). However, sex-specific responses to excess Zn stress and neighbor effects have been largely overlooked in *P. cathayana*, especially in relation to rhizospheric microbial community structures. In the present study, *P. cathayana* was used to examine sexual differences in competitive ability under excess Zn conditions. We aimed to address the following questions: (i) Does excess Zn affect intra- and intersexual interactions? (ii) How do intra- and intersexual interactions affect rhizospheric bacterial communities? (iii) Do interactive effects between excess Zn and sexual interactions affect plant growth and rhizospheric bacterial community composition and diversity?

2. Materials and methods

143 *2.1. Plant materials and experimental design*

144

145 Cutting of *P. cathayana* females and males were collected from 60 different trees
146 sampled in 15 populations in the riparian and valley flat habitats of the Qinghai
147 Province, China. Cuttings were rooted as described by Chen et al. (2016). The
148 experimental design was completely randomized with three factors (sex, excess Zn and
149 plantation type), i.e. two sexes (females, males), two Zn regimes (no-Zn, Zn) and five
150 plantation types. After sprouting and growing for 4 weeks, uniform seedlings were
151 planted into 60-L plastic pots filled with *c.* 40 kg of homogenized soil. The soil was
152 collected from the poplar plantation at the Hangzhou Normal University, Zhejiang
153 Province, China (30.03' N, 120.12'E). Soil samples were air-dried and sieved through
154 2 mm sieve. The properties were as follows: 1.77 g kg^{-1} total N, 1.80 g kg^{-1} total P, 7.91
155 g kg^{-1} total K, and 0.1 g kg^{-1} total Zn. For the excess Zn treatment, 1 L deionized water
156 containing 100 μM $ZnSO_4$ was used to evenly irrigate the pots every two days until the
157 final Zn level of 50 mg $ZnSO_4$ kg^{-1} dry soil was reached, while the control plants were
158 irrigated with equal quantities of deionized water (Chen et al., 2016).

159

160 The five plantation types were as follows: F, single-cultivated females; M, single-
161 cultivated males; FF, female × female; MM, male × male; FM, female × male.
162 Intrasexual neighboring plants were denoted as M/MM for males and F/FF for females.
163 Intersexual neighboring plants were denoted as M/FM for males and F/FM for females.
164 For single-plant cultivation, one cutting was planted per pot (a female or a male); for

interactions, two plants (two females, two males or a female and a male) were cultivated 10 cm apart from each other in a plastic pot (external diameter 52 cm and height 35 cm). All pots were arranged randomly and each treatment was replicated four times. The experiment was performed at the Hangzhou Normal University. The plants were cultured for 120 d in a semi-controlled greenhouse at the Hangzhou Normal University (China, 30.03' N, 120.12'E) with a relative humidity of 76%-81%, a daytime temperature of 21-25 °C, a night-time temperature of 15-18 °C and a photoperiod of 12-14 h throughout the growth period.

2.2. Gas exchange and fluorescence measurements

The fully developed young leaves from each plant were used to measure gas exchange and chlorophyll fluorescence. Net CO_2 assimilation rate (A) and stomatal conductance (g_s) were measured with a portable photosynthesis measuring system (Ll-6400), as described previously by Chen et al. (2011). Chlorophyll fluorescence kinetics parameters (*ETR*, electron transport rate; quantum yield of photochemical energy conversion in *PS II*, *Y(II)*; quantum yield of regulated non-photochemical energy loss in *PS II*, *Y (NPQ)*; F_v/F_m, variable and maximum fluorescence) were measured with a PAM chlorophyll fluorometer (PAM 2100, Walz, Effeltrich, Germany). These parameters were calculated according to the method of Van Kooten and Snel (1990).

2.3. Soil sampling, plant harvesting and element measurements

After the measurement of photosynthesis, rhizosphere soil was collected by gently shaking a plant root; the soil adhering to the root was collected as a sample of rhizospheric soil. In total, 40 soil samples (4 replicates × 5 interaction patterns × 2 treatments) were immediately sieved (4 mm) in the laboratory. The rhizospheric soils were divided into two subsamples. One subsample was oven dried at 75 ºC and used for analyzing soil properties, and another subsample was stored at -80 ºC until DNA extraction.

The plants were separated into leaves, stems and roots, and washed with deionized water. Dried leaves and roots were finely ground, and about 0.4 g samples (< 1 mm) were dissolved in 3:1 (v/v) of HNO_3 and $HClO_4$. The mixtures were carefully shaken and predigested at room temperature for 30 min. The vessels used for digestion were sealed and placed into a microwave digestion system. The microwave heating program was performed as follows: (1) 10 min at 170 ºC; (2) 10 min at 190 ºC; (3) 10 min at 210 ºC (Tokalıoğlu et al., 2018). The vessels were cooled down to room temperature to avoid splashing and foaming. Clear digested solutions were transferred to 25 ml polyethylene tubes, which were filled to a volume of 25 ml with ultra-pure water for further ICP-MS analysis. Total Zn and nutrient elements were measured with ICP-MS (inductively coupled plasma mass spectrometer; Agilent 7500a; Agilent Technologies). For quality assurance/quality control (QA/QC) purposes, a blank control and standard reference material GBW10020 (GSB-11) of citrus leaves were used to validate quantification according to Xie et al. (2020). Blank control and reference materials were

210 treated under the same conditions as the experimental samples. The recovery of the
211 standard at a medium calibration level was checked with every 10 samples. The method
212 detection limits (mg kg^{-1}) were 0.2 mg l^{-1} for Zn and the recovery rates were 80-90%.
213 All data are presented as means ± the standard deviations, unless specified differently.
214
215 The translocation factor (T_f) was defined as the Zn concentration in a shoot divided by
216 the Zn concentration in roots (He et al., 2013b). The bio-concentration factor BCF was
217 calculated as the Zn concentration in roots or shoots divided by the Zn concentration in
218 the soil (Shi et al., 2010; He et al., 2013b).
219
220 *2.4. Statistical analysis*
221
222 Statistical analyses were carried out using the SPSS software package (version 22.0).
223 All data were checked for normality before analyses of variance (ANOVAs).
224 Differences between means were analyzed by Duncan's tests following three-way
225 ANOVAs, which were used to evaluate sexual interaction and excess Zn treatment
226 effects.
227
228 *2.5. DNA extraction, 16S amplification and MiSeq sequencing*
229
230 Approximately 0.5 g of each soil sample was used to extract genomic DNA utilizing
231 the PowerSoil DNA Isolation Kit (MoBio Laboratories, Inc. Carlsbad, USA) following

232 the manufacturer's instructions. The 16S rRNA genes were amplified using the primer
233 pair 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-
234 GGACTACHVGGGTWTCTAAT-3'). Approximately 10 ng of purified DNA was
235 amplified with 25 μl of the PCR reaction system under the following conditions: at
236 95 °C 2 min for denaturation, followed by 40 cycles of 10 s at 95 °C for denaturation,
237 30 s at 56 °C for annealing, and 20 s at 72 °C for extension, with 55 to 95 °C for melting
238 curve analyses. The PCR products were pooled and purified with a MicroElute Cycle-
239 Pure Kit (Omega), and high-throughput sequencing was performed using the Hiseq
240 2500 PE250 platform with 2×250 bp according to the manufacturer's manual.
241
242 *2.6. Processing of high-throughput sequencing data and analysis*
243
244 Amplicon sequences were processed using the QIIME 2 version 2017.12
245 (https://qiime2.org/). All sequences of 16S rRNA raw data were demultiplexed, and
246 quality control was carried out using DADA2 with the "consensus" method, to remove
247 chimeric and low-quality sequences (Callahan et al., 2017; Yuan et al., 2018). When the
248 paired-ends were joined, the unreliable and low-quality sequences were detected based
249 on the low sequence quality of the 3′-ends of the reads (Merloti et al., 2019). After that,
250 the Amplicon Sequence Variants (ASVs) were created using the Deblur tool. The
251 resulting final ASV table contained only high-quality reads. The taxonomic
252 identification of ASVs (with 99% of similarity) was conducted with the VSEARCH
253 consensus taxonomy classifier implemented in Qiime2 and the SILVA 16S rRNA

254 database. The archaeal and chloroplast sequences were removed. Statistical analyses of
255 the 16S rRNA microbiome sequencing data were performed using the generated
256 taxonomic matrices. The sequencing data were submitted to NCBI (BioProject
257 accession number: PRJNA644210).
258
259 *2.7. Statistical analysis of sequencing data*
260
261 The α-diversity of bacteria was characterized by the Simpson's diversity index and the
262 effective number of species, and the data were analyzed with ANOVA using Duncan's
263 test ($P < 0.05$). β-diversity was calculated based on weighted-UniFrac distance metrics.
264 Principal coordinates analysis (PCoA) was used to separate the overall bacterial
265 community structure based on the weighted-UniFrac distance with the "pcoa" function
266 of the "ape" package in R (v3.2.2). Permutational multivariate analysis of variance
267 (PERMANOVA) was performed to separate and evaluate the effects of sex, plantation
268 mode and Zn treatment and theirinteractions on the rhizospheric bacterial communities
269 using the "anosims" function in "vegan" package in R (v3.2.2). The linear discriminant
270 analysis (LDA) effect size (LEfSe) algorithm was used to analyze the relative bacterial
271 abundance in different treatments, irrespective of sexual interactions (Class: Zn
272 treatment; Subclass: interaction patterns), as well as in different sexual interaction
273 patterns irrespective of Zn treatment (Class: sexual interaction; Subclass: Zn treatment).
274 The online Galaxy application (version 1.0) was used with a threshold of 1.0 and a
275 Wilcoxon *P*-value of 0.05 (http://huttenhower.sph.harvard.edu/galaxy/). A similarity

percentage (SIMPER) analysis was performed with the PRIMER 6 software to find differences between sexual interaction patterns and other treatments. The OTUs less than 1% of the relative abundance were discarded from the analysis according to Marasco et al. (2018). Correlation coefficients between the bacterial abundance at the phyla level and Zn amount were obtained with python (SciPy package). The heatmap of correlation coefficients was plotted with R (Heatmap package).

2.8. PICRUSt functional prediction

The *PICRUSt* (*phylogenetic investigation of communities by reconstruction of unobserved states*, v1.0.0) pipeline was used to predict the relative abundance of gene transcripts in bacteria. The *pick_closed_reference_otus.py script* in QIIME (similarity threshold, 0.97) was used to cluster sequences into OTUs. The OTUs were first normalized by the copy number by removing the copy number of the 16S marker gene. The Nearest Sequenced Taxon Index (NSTI) and KEGG Ortholog (KO) were obtained by the *predict_metagenomes.py* script. The KO level 3 within the pathway hierarchy of KEGG was collapsed using the *categorize_by_function.py* script (Langille et al., 2013; Hou et al., 2017). The predicted metagenomes were then annotated using the KEGG database, and the predicted relative abundances of gene transcripts for selected pathways associated with bacterial functions were analyzed and plotted using STAMP (Parks et al. 2014; Hou et al. 2017).

3. Results

298

299 *3.1. Plant growth and Zn phytoextraction*

300

301 Under control conditions (no excess Zn), males showed 21% and 48% higher CO_2
302 assimilation rate (A) and stomatal conductivity (g_s) respectively, than females under
303 single-plant cultivation (Table 1). Females had lowest A and g_s under intersexual
304 interaction (F/FM)under control conditions (Table 1). Compared with controls, A and
305 g_s decreased by 29% and 18%, respectively, in females and by 19% and 22%,
306 respectively, in males in single-sex cultivation under excess Zn (Table 1). Excess Zn
307 decreased A by 18%, 11%, 12% and 16%, and g_s by 20%, 22%, 32% and 57%, in F/FM,
308 M/FM (males in intersexual interaction), F/FF (females in intrasexual interaction) and
309 M/MM plants (males in intrasexual interaction), respectively (Table 1). In addition,
310 M/FM exhibited 10% and 55%% increases in A and g_s compared to M/MM under
311 excess Zn conditions (Table 1).

312

313 Excess Zn treatment decreased F_v/F_m, *ETR* and *Y (II)* by 6%, 37% and 50%,
314 respectively, in females and by 4%, 17% and 8%, respectively, in males under single-
315 sex cultivation (Table 1). Sexual interactions did not affect F_v/F_m in either sex under
316 control conditions (Table 1). Excess Zn did not affect F_v/F_m and *ETR* in males (M/MM)
317 and females (F/FF) when compared with controls. However, F_v/F_m, *ETR* and *Y (II)* were
318 4%, 37% and 54% higher, respectively, in M/FM plants than in F/FM plants under
319 excess Zn, and *Y (II)* of males was 13% higher when compared to females under

320 intrasexual interaction (Table 1). In addition, excess Zn treatment increased *Y (NPQ)*
321 by 71% in females, but not in males, under single-plant cultivation (Table 1) when
322 compared to control conditions. Sexual interactions did not affect *Y (NPQ)* in either sex
323 under control conditions. However, excess Zn increased *Y (NPQ)* by 32%, 58% and 14%
324 in F/FM, F/FF and M/MM, respectively, when compared to controls (Table 1).
325
326 Excess Zn increased the Zn content in by 22.9- and 4.3-fold, respectively, in leaves,
327 by 3.1- and 1.4-fold, respectively, in shoots, and by 2.7- and 3.2-fold, respectively, in
328 roots of females and males under single-sex cultivation (Fig. 1). Under excess Zn
329 conditions, Zn levels increased by 4.6-, 29.6-, 13.6- and 13.1-fold, respectively, in
330 leaves, by 97%, 300%, 327% and 219%, respectively, in stems, and by 160%, 240%, %
331 and 160%, respectively, in roots of F/FM, M/FM, F/FF and M/MM when compared to
332 controls (Fig. 1). Under excess Zn, the Zn level of females was 37% and 39% higher in
333 leaves and roots, respectively, but 82% lower in stems when compared to males in
334 single-sex cultivation under excess Zn conditions. The leaf and stem Zn levels of males
335 were lower than those of females under intersexual interaction; the contrary was true
336 for intrasexual interaction under excess Zn (Fig. 1). In addition, there was no difference
337 in root Zn concentrations between sexes under intersexual interaction, whereas the Zn
338 level in roots of M/MM was 26% lower than that of F/FF plants.
339
340 Females had higher T_f (the ratio of Zn in the shoots to roots) than males under single-
341 sex cultivation. T_f was higher in both sexes under single-sex cultivation than under

intersexual interactions (except for F/FF) when exposed to excess Zn (Fig. 1). F/FM had highest T_f and F/FF showed the lowest T_f among all excess Zn treatments (Fig. 1). Under excess Zn, M/MM showed 16% higher T_f than M/FM. In single-sex cultivations under excess Zn, females had 141% and 98% increases, respectively, in root BCF (the ratio of Zn in the roots to soil) and shoot BCF (the ratio of Zn in the shoots to soil) when compared to males. F/FF had highest root BCF among all excess Zn treatments. Root and shoot BCF values were48% and 72% higher, respectively, in M/MM than in M/FM under excess Zn, while females had 1.2-fold higher shoot BCF than males under intersexual interaction with excess Zn (Fig. 1).

3.2. Dominant taxa of bacterial communities

The bacterial communities predominantly consisted of Proteobacteria (33%), Actinobacteria (17%), Acidobacteria (16%), Chloroflexi (14%), Planctomycetes (6%), Bacteroidetes (4%), Gemmatimonadetes (2%), Crenarchaeota (2%), Verrucomicrobia (1%), and Nitrospirae (1%). These dominant species were affected by plant interactions and excess Zn (Fig. 2). Excess Zn reduced the relative abundance of the Proteobacteria phylum in the rhizosphere of M/MM and FM, but did not affect Proteobacteria in the rhizosphere of F/FF. Excess Zn reduced the abundance of Actinobacteria and Planctomycetes phyla in both sexes under intersexual interactions. The relative abundance of Proteobacteria and Actinobacteria phyla were higher under the excess Zn treatment than under control conditions in both sexes when plants were cultivated alone,

364 while the contrary was true for the Chloroflexi phylum (Fig. 2).

365

366 *3.3. Bacterial community diversity and structure*

367

368 The alpha-diversities of bacterial communities (effective numbers of species) were
369 compared in females and males under different plantation modes and excess Zn
370 treatments. As shown in Fig. 3, intra- and intersexual interaction affected bacterial
371 community structures, but the effects of these interaction patterns were greater under
372 excess Zn. Permutational multivariate analysis of variance (PERMANOVA) further
373 demonstrated that plantation modes were the largest source of variation (25.16%, $P <$
374 0.001; Table 2). The Zn levels were the second largest source of variation (5.00%, $P <$
375 0.004; Table 2).

376

377 *3.4. Taxonomic composition of bacterial communities*

378

379 The linear discriminant analysis (LDA) effect size analysis (LEfSe) was performed to
380 compare the bacterial composition from phyla to genera between Zn treatments, as well
381 as between sexes (Fig. 4). We found that bacterial compositions showed significant
382 differences among sexual interaction patterns and Zn treatments. The orders
383 Acidimicrobiales, Micromonosporaceae, RB40, Verrucomicrobiales, Thiotrichales,
384 MND1, Piscirickettsiaceae and Pirellulaceae were predominant under excess Zn, while
385 the genera *Pontibacter*, *Chryseobacterium*, *Lysobacter*, *Kaistobacter* and *Massilia*, and

386 the class Flavobacteria were enriched in plants without excess Zn under single
387 cultivation (Fig. 4a). Moreover, the order Sva0725, the family A4b and the genus
388 *Rubrivivax* were dominant in the rhizosphere of males, while other taxa were enriched
389 in soil with females (Fig. 4b).
390
391 In sexual interaction experiments, the order Nitrososphaerales was generally more
392 abundant in the soil of control plants (Fig. 4c). Under excess Zn, the class Saprospirae,
393 the orders GCA004, Solirubrobacterales and AKYG1722, the families *AKIW874*,
394 *Cellulomonadaceae*, *Mycobacteriaceae* and *Sporichthyaceae*, and the genera *Bacillus*,
395 *Rhodoplanes* and *Mycobacterium* were predominant (Fig. 4d). Irrespective of the Zn
396 treatment, the genera *Agromyces* and *Mycobacterium*, as well as the family
397 *Mycobacteriaceae* were abundant in the soil of M/MM, whereas the order
398 Methanobacteriales and the genus *Methanothermobacter* were more abundant in the
399 soil of F/FF under intrasexual interaction (Fig. 4d). In contrast, orders Caldilineales and
400 NB1-j were more prevalent in the soil of FM exposed to intersexual interaction (Fig.
401 4d).
402
403 *3.5. Functional predictions of bacterial communities*
404
405 The PICRUSt analysis was performed to predict the metagenome gene functions. As
406 shown in Fig. 5, the PICRUSt analysis suggested that the predicted differential gene
407 abundances between treatments were related to carbohydrates, amino acids, lipids and

408 secondary metabolism in the rhizosphere of females and males under single-plant
409 cultivation. When compared to females under single-plant cultivation, males had a
410 higher abundance of predicted genes involved in the citrate cycle and carotenoid
411 biosynthesis in the rhizosphere in control conditions (Fig. 5a). Excess Zn stress reduced
412 arachidonic acid metabolism, flavonoid biosynthesis and peptidoglycan, and increased
413 the abundance of predicted genes related to glycolysis, citrate cycle and leucine
414 synthesis in females under single-plant cultivation.
415
416 Under plant interactions, the differentially abundant genes in different treatments in the
417 rhizospheres of both sexes were related to amino acid translation, and amino acid and
418 lipolic acid metabolism under single-plant cultivation (Fig. 5b). Excess Zn reduced the
419 abundance of the predicted genes related to cysteine, methionine and thiamine
420 metabolism, especially in the rhizosphere of females exposed to intrasexual interaction.
421 However, excess Zn elevated the abundance of predicted genes related to tyrosine
422 metabolism in the rhizosphere of females, compared to males, both under inter- and
423 intraspecific interaction (Fig. 5b).
424
425 *3.6. Relative abundance of key taxa associated with Zn levels in plants*
426
427 We examined the bacterial abundance in the rhizosphere at the genus level in relation
428 to Zn levels in roots (Fig. 6). First, we examined the relative abundance of taxa
429 positively associated with Zn levels. Under single-plant cultivation, females showed a

relatively higher abundance of families Streptomycetaceae and Rhodobacteraceae, and the phylum Chloroflexi than males under an increasing Zn supply in the rhizosphere. In contrast, the increased Zn level raised the relative abundance of the family Sphingomonadaceae in the rhizosphere of males compared to the female rhizosphere. Under intrasexual interaction, males had a higher relative abundance of the Actinomycetales order and Phyllobacteriaceae family when compared to intersexual interaction (Fig. 6). By contrast, the family Alphaproteobacteria was more abundant in the rhizosphere of males exposed to excess Zn under intersexual interaction than under intrasexual interaction. In females, the orders Myxococcales, Roseiflexales and Actinomycetales, and the class Betaproteobacteria were predominant in the rhizosphere exposed to intersexual interaction under excess Zn relative to intrasexual interaction (Fig. 6). The increased abundance of the order Rhizobiales under excess Zn was specific to females exposed to intrasexual interaction relative to intersexual interaction. Under intersexual interaction, the abundance of the order Actinomycetales was higher in males, while the order Rhizobiales was more abundant in the rhizosphere of females under excess Zn (Fig. 6).

Then, we examined the relative abundance of bacteria negatively associated with Zn levels in roots (Fig. 6b). We found that under excess Zn, the relative abundance of most bacterial classes, such as Planctomycetia, Betaproteobacteria and Acidobacteria-6, were lower in the rhizosphere of females than in that of males under single-sex conditions. When compared to females under single-sex conditions, males had a higher

abundance of the family Hyphomonadaceae and the order WD2101 irrespective of the Zn application. Under intrasexual interaction with excess Zn, the abundance of the class Gemm-5 in the rhizosphere of males, and the abundance of the genus *Planctomyces* in females and males were higher than under intersexual interaction (Fig. 6b). On the other hand, under intersexual interaction with excess Zn, the abundance of the RB40 family was higher but the abundance of the genus *Planctomyce*s was lower in the rhizosphere of females than in males.

4. Discussion

4.1. Sexual interactions affect Zn phytoextraction in plants

Neighbor interactions of plants affect plant growth and community composition (Hodge and Fitter, 2013; Hawkins and Crawford, 2018). In turn, these interactions could be altered by abiotic stress, e.g. heavy metals (Chen et al., 2016, 2017). It has been shown that abiotic stress can alter competitive interactions (Chen et al., 2016, 2017). Selenium has been found to strongly influence plant-plant interactions and play a vital role in elemental allelopathy (El Mehdawi et al., 2011). In this study, under excess Zn stress, F/FM accumulated more Zn in their leaves and barks compared to F/FF, while males accumulated less under intersexual interaction than under intrasexual interaction (Table 1; Fig. 1). Responses of sexes to abiotic stresses depend mainly on the specific properties of stresses, the soil status and the exposure duration (Howard et al., 2000;

474 Tonneijck et al., 2004). Zn is an essential trace element for plants and, in our study, the
475 leaf Zn levels did not reach the toxic threshold for poplars under any interaction (Fig.
476 1). Females and males exposed to inter- and intrasexual interaction showed strong
477 tolerance to excess Zn (Table 1). Interestingly, we found that under intrasexual
478 interaction both females and males accumulated more Zn in the roots, which largely
479 inhibited Zn transportation to the shoots and leaves (Fig. 1). The reduced heavy metal
480 accumulation in leaves facilitates heavy metal tolerance in woody plants (He et al.
481 2013a).
482
483 The tolerance of plants to heavy metals is also reflected in leaf photosynthesis (Chandra
484 and Kang, 2016; Salisbury et al., 2018), which indicates the ability of plants to thrive
485 in specific environments, including heavy metal stress(Simkin et al., 2019; Hu et al.,
486 2020). The accumulation of heavy metal in leaves damages photosynthetic electron
487 transport and reduces photosynthesis, which are signs of stress (Aggarwal et al., 2012).
488 We found that females had higher Zn enrichment but lower Zn toxicity tolerance than
489 males under Zn treatments. The inhibited photosynthesis and electron transmission
490 efficiency were associated with Zn translocation into leaves (Fig. 1; Table 1). Females
491 had higher root-to-shoot translocation under intrasexual than intersexual interaction, as
492 visible as elevated root BCF but lower T_f under excess Zn (Fig. 1). However, males
493 favored to accumulate more Zn in roots, showing higher root BCF but lower shoot BCF
494 (Fig. 1). Moreover, females had a higher Zn translocation efficiency (T_f) under
495 intersexual than intrasexual interaction, while the contrary was true for males (Fig. 1).

Moreover, the Zn translocation efficiency was higher in females than in males when subjected to excess Zn under intersexual interaction. In previous studies, female plants have showed higher competitive ability than males (Mercer and Eppley, 2010; Sánchez-Vilas et al., 2011; Chen et al., 2016). Consistently, we found that females had a stronger competitive ability under excess Zn even when excess Zn was toxic for plants. In a previous study, we found that females had a higher expression of genes, such as those from the ZIP family, which facilitate Cd uptake and root-to-shoot translocation in *P. cathayana* (Liu et al., 2020b). Importantly, the potential of females and males for extracting Zn also depends on underground processes that affect Zn availability (Fig. 6).

4.2. Soil microbial composition modified by plantation modes and Zn pollution

Heavy metals affect rhizosphere-driven microbial community structures (Hou et al., 2017). Soil microorganisms are also critical drivers in modifying community structures and plant-plant interactions (Hodge and Fitter, 2013; Hawkins and Crawford, 2018). The sexually differential trade-off between plant rewards and defense has been shown to influence the colonization of host-specific microbial communities (Bever et al., 2012; Varga et al., 2017). This finding was further demonstrated by our study. We discovered that the bacterial community diversity and richness in the rhizosphere of both sexes were not affected by plantation modes or excess Zn, but probably associated with the planting time and species (Siciliano et al., 2014; Tian and Gao, 2014; Fontana et al.,

518 2016). However, the bacterial community structure was strongly affected by plantation
519 modes and Zn treatments (Fig. 3), which probably affected root Zn bioavailability.
520
521 Generally, heavy metals suppress the growth and abundance of low-resistance microbes
522 but increase the prevalence of high-resistance bacteria (Pishchik et al., 2016; Wood et
523 al., 2016; Hou et al., 2017). Consistently, the relative abundance of Cytophagales was
524 significantly inhibited by excess Zn in both sexes, while the abundance of key phyla,
525 such as Betaprteovacteria, Acidoacteria, Nitrosopirales and Proteobacteria, increased
526 under excess Zn in single-cultivated males (Fig. 4; Figs S1-S2). Interestingly, the
527 abundance of Betaprteovacteria, Acidoacteria, Nitrosopirales and Proteobacteria phyla
528 were positively associated with root Zn levels (Fig. 6), which suggested that they are
529 probably involved in excess Zn uptake. In addition, we found that when compared to
530 untreated soil, Zn levels in excess Zn-treated soil were 3~7 -fold higher (Fig. S5), and
531 the level was higher than the Zn toxicity risk screening value for soil (Huang, 2014;
532 Carruthers, 2016). Our results support the view that phytoremediation may be an
533 effective strategy to improve the soil quality by recruiting some beneficial microbes
534 (Ancona et al., 2020). It is evident that if attempting to use microbial remediation of
535 metals, it would be important to investigate further the responses of specific bacterial
536 families.
537
538 Sexual interactions alter the structure of root systems and the secretion of root exudates
539 in *P. cathayana* females and males, thus modifying the rhizosphere ecology (Ke and

Wang, 2020; Xia et al., 2020). The present study found that the Methanotheriales, Mycobacterium and Caldilineales phyla were enriched in the rhizosphere of F/FF, M/MM and FM, respectively (Fig. 4). Among these specific bacteria, the Actinobacteria and Streptomyces phyla have been identified as the most dominant taxa in soils with heavy metal contamination (Watve et al., 2001; Ellis et al., 2003; Alvarez et al., 2017), and they became more abundant under excess Zn in the rhizosphere of M/MM and M/FM (Fig. 4; Figs S3-S4). More importantly, the relative abundance of Actinobacteria showed a positive correlation with root Zn levels in M/MM and FM (Fig. 6). Actinobacteria produce siderophores for Cd acquisition and protect plants from the invasion of pathogenic bacteria (Viaene et al., 2016). The specific colonization of Actinobacteria in the rhizosphere of *Arabidopsis* confers a competitive advantage to these plants (Van der Meij et al., 2018). The increased abundance of Actinobacteria following the excess Zn treatment in the rhizosphere of FM was probably due to Zn chelation, which was likely the result of a long-term adaptation of sexes to excess Zn under intersexual interaction. *Streptomyce*s is the largest genus producing antibiotics (Watve et al., 2001; Hong et al., 2009). It has been suggested that some strains belonging to *Streptomyces* promote metal solubility and auxin synthesis by stimulating siderophore synthesis (Zloch et al., 2016; Hou et al., 2017). Taking into account the higher Zn accumulation in females under single-sex and intersexual modes (Fig. 1), Streptomycetaceae probably plays a key role in the Zn accumulation in *P. cathayana* females under excess Zn.

562 We also found some sex-specific bacterial colonization in the *P. cathayana* rhizosphere
563 under different interaction patterns. The excess Zn treatment increased the abundance
564 of the phylum Euryarchaeota in both females and males with intrasexual interaction
565 (Figs S3-S4). In addition, the abundances of the phyla Actinobacteria and Chloroflexi
566 were elevated under excess Zn in the rhizosphere of FM and M/MM (Fig. 4; Figs S3-
567 S4). Overall, our results suggest that most bacteria prefer to colonize the rhizosphere of
568 both sexes in the case of intersexual interaction, irrespective of Zn levels, as well as in
569 the rhizosphere of males exposed to intrasexual interaction.
570
571 *4.3. Microbial functional prediction highlights the role of sexual interaction patterns*
572
573 The PICRUSt analysis suggested that excess Zn and sexual interactions differentially
574 regulate the abundance of predicted gene transcripts of bacteria in the rhizosphere. The
575 excess Zn treatment increased the abundance of predicted gene transcripts related to
576 amino acids and organic acids, such as valine, leucine and isoleucine biosynthesis, and
577 the citrate cycle in both sexes under single-plant cultivation, which probably increased
578 organic matter from dead roots, exudates and rhizodeposits (Hou et al., 2017, 2018). It
579 is worth noting that the abundance of predicted gene transcripts related to carotenoid
580 biosynthesis was higher in the rhizospheric bacteria of males than in those of females
581 (Fig. 5a), which probably played an important role in heavy metal tolerance. Excess Zn
582 increased the abundance of predicted gene transcripts related to tyrosine metabolism
583 and secondary metabolites in males exposed to intersexual interaction and in females

584 under both intra- and intersexual interaction, suggesting that excess Zn differentially
585 regulates the abundance of predicted gene transcripts. Further studies are needed to
586 explore the relationship between heavy metal stress and gene expression among the
587 bacteria in the rhizospheres of both sexes.
588
589
590
591 **5. Summary**
592
593 This study suggested that excess Zn and sexual interactions strongly affect Zn
594 accumulation, and microbial abundance and diversity. Females have higher Zn
595 accumulation and root-to-shoot translocation under intersexual than under intrasexual
596 interaction combined with excess Zn; the contrary was true for males. Moreover, the
597 plantation modes and excess Zn treatment altered the bacterial structure of the
598 rhizosphere, which largely affected the Zn availability and uptake of roots. The Zn-
599 polluted soil with males growing under different interactions promoted some key
600 bacterial taxa related to metal activation and chelation in the rhizosphere, e.g.,
601 Actinobacteria and Streptomyces, while the excess Zn-treated soil with females had a
602 lower bacterial abundance under intrasexual interaction than under intersexual
603 interaction. To our knowledge, this is the first study that has showed a possible
604 relationship of Zn availability with specific bacterial colonization in the rhizosphere of
605 *P. cathayana* females and males under sexual interactions. Our study provides new

606 insight into the interactions among plant sex, plantation types, heavy metal stress and
607 the composition of microbial communities.
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613
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616
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618 analysis and writing, Yuting Wang and Xiucheng Liu contributed to data collection,
619 Helena Korpelainen contributed to the interpretation of data and manuscript preparation,
620 and Chunyang Li (the corresponding author) had the overall responsibility for
621 experimental design and project management.
622
623 **Conflict of interest** The authors declare that they have no conflict of interest.
624
625 **Appendix A. Supplementary data** Supplementary material related to this article can
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Figure legends

Fig 1. Zinc (Zn) accumulation in leaves (a), stems (b) and roots (c), translocation factor (T_f) (d), the root bio-concentration factor (BCF) (e) and shoot BCF (f) in *P. cathayana* females and males under different cultivation patterns and excess Zn. Values are expressed as means ± SD (n = 4). Different letters represent significant differences between treatments ($P < 0.05$). F, female; M, male; F/FF, female under intrasexual interaction; M/FM, male under intersexual interaction; M/MM, male under intrasexual interaction; F/FM, female under intersexual interaction.

Fig 2. Taxonomic compositions of bacterial communities in the rhizospheres of *P. cathayana* females and males at the phylum level with the relative abundance over 1% under excess zinc (Zn). Rhizospheric soil samples from plants under single-sex (a) and double-sex interactions (b). Average relative abundance of key phyla in the rhizospheric soil from plants under single-sex (c) and double-sex interactions (d); FF, female with intrasexual interaction; FF+Zn, FF with excess Zn; FM, female and male under intersexual interaction; FM+Zn, FM with excess Zn; MM, male with intrasexual interaction; MM+Zn, MM with excess Zn. A, Proteobacteria; B, Actinobacteria; C, Chloroflexi; D, Acidobacteria; E, Planctomycetes; F, Bacteroidetes; G, Gemmatimonadetes.

Fig 3. Box plots for alpha diversity (effective number of species) (a, b) and beta-

diversity (principal co-ordinates analysis) of bacteria from the rhizospheric soil of *P. cathayana* females and males under excess zinc (Zn) treatment. Alpha diversity of bacteria from the rhizospheric soil of plants under single-sex (a) and double-sex interaction (b). Principal coordinate analysis plots among bacterial communities in the rhizosphere of *P. cathayana* females and males under single-sex interactions (c) and double-sex interactions (d). F/FM, female under intersexual interaction; M/FM, male under intersexual interaction; FF, female under intersexual interaction; MM, male under intrasexual interaction.

Fig 4. LEfSe used to identify abundant taxa in the rhizospheric soil from *P. cathayana* females and males under excess zinc (Zn) treatment. A cladogram was generated by LEfSe indicating differences between bacteria at phylum, class, family and genus levels under single-plant cultivation between excess zinc (Zn) and control soil (no-Zn treatment), irrespective of sex and interaction (a), and between sexes, irrespective of Zn treatment (b). Another cladogram was generated by LEfSe indicating differences between bacteria at phylum, class, family and genus levels between excess Zn and control soil, irrespective of sex and interaction (c), and between interaction modes, irrespective of Zn treatment (d). The node colour indicates taxa enriched under different treatment and interaction patterns. Only taxa with LDA over 3 are shown. FF, female under intrasexual interaction; FM, female and male under intersexual interaction; MM, male under intrasexual interaction.

962 **Fig 5.** Predicted functions of the bacterial communities from the rhizospheric soil of *P.*
963 *cathayana* females and males under excess zinc (Zn) treatment. Predicted functions of
964 the bacterial communities from the rhizospheric soil of plants from single sex (a) and
965 double-sexual interactions (b). F, female; F+Zn, F with excess Zn; M, male; M+Zn, M
966 with excess Zn; F/FM, females under intersexual interaction; M/FM, male under
967 intersexual interaction; FF, female under intrasexual interaction; FF+Zn, FF with excess
968 Zn; MM, male under intrasexual interaction; MM+Zn, male under intrasexual
969 interaction.
970
971 **Fig 6.** Heatmaps of the average relative abundance of key bacteria at the genus level
972 positively (a) or negatively associated (b) with Zn levels in roots of *P. cathayana*
973 females and males exposed to different interaction patterns ($P < 0.05$). F, female; M,
974 male; FF, female exposed to intrasexual interaction; M/FM, male exposed to intersexual
975 interaction; MM, male exposed to intrasexual interaction; F/FM, female exposed to
976 intersexual interaction.

Table 1 Net photosynthesis rate (*A*), stomatal conductance (g_s), and fluorescence parameters *Fv/Fm*, *ETR*, *Y (II)* and *Y (NPQ)* of *P. cathayana* females and males exposed to different sexual interactions under control conditions or excess zinc (Zn). F_v/F_m, maximum quantum efficiency of *PS II* photochemistry; *ETR*, electron transport rate; *Y(NPQ)*, quantum yield of regulated non-photochemical energy loss in *PS II*; *Y(II)*, quantum yield of photochemical energy conversion in *PS II*.

Treatment	***A* ($\mu mol\ m^{-2}\ s^{-1}$)**	***g*$_s$ ($mol\ m^{-2}\ s^{-1}$)**	**F_v/F_m**	***ETR***	***Y (II)***	***Y (NPQ)***
Control						
F	19.21±0.35d	0.24±0.02f	0.79±0.01cd	51.48±0.52abc	0.45±0.04bc	0.28±0.01fg
M	23.31±1.41a	0.36±0.03b	0.81±0.00a	52.88±2.85ab	0.44±0.02cd	0.33±0.01de
F/FM	19.11±0.27d	0.29±0.00de	0.80±0.01bc	49.10±3.12cd	0.44±0.02cd	0.27±0.11fg
M/FM	22.49±0.26b	0.41±0.01a	0.80±0.01abc	54.4±2.20a	0.49±0.01a	0.26±0.01fg
F/FF	22.15±0.93ab	0.36±0.01b	0.80±0.01bc	51.93±1.86abc	0.48±0.02ab	0.28±0.02f
M/MM	21.64±0.48b	0.34±0.03bc	0.81±0.01ab	54.83±2.35a	0.50±0.01a	0.28±0.01fg
Zn treatment						
F	13.73±0.40f	0.20±0.01g	0.75±0.02f	32.23±2.21g	0.23±0.01i	0.49±0.02a
M	18.94±0.01e	0.28±0.03e	0.78±0.00d	43.78±1.12e	0.40±0.02e	0.35±0.01cd
F/FM	18.11±0.27e	0.23±0.02fg	0.76±0.01e	36.88±3.59f	0.27±0.04h	0.36±0.04c
M/FM	20.12±0.26c	0.32±0.02cd	0.80±0.01bcd	50.68±1.92bc	0.41±0.02de	0.25±0.03g
F/FF	19.77±0.86cd	0.25±0.04f	0.79±0.00d	44.33±1.36e	0.32±0.03g	0.45±0.04b
M/MM	18.15±0.10e	0.15±0.02h	0.78±0.01d	47.00±1.43de	0.36±0.01f	0.32±0.02e

F, female; M, male; FF, female exposed to intrasexual interaction; M/FM, male exposed to intersexual interaction; MM, male exposed to intrasexual interaction; F/FM, female exposed to intersexual interaction; Different letters represent significant differences between treatments ($P < 0.05$). Values are means ± SE ($n = 4$).

Table 2 PERMANOVA results using Bray-Curtis as a distance metric.

Factor	% explained	*F*	R^2	*P*
Plantation modes	25	4.38	0.25	0.001***
Zn	5	3.49	0.05	0.004**
Sex	5	3.19	0.05	0.006**
Sex : Zn	2	1.28	0.02	0.227
Plantation modes : Zn	11	1.91	0.11	0.007**
Residuals	52		0.52	

Plantation modes: F, M, F/FF, FM, M/MM; sex: F, M; Zinc, excess Zn treatment. * $0.01<P \leq 0.05$; ** $0.001<P \leq 0.01$; *** $P \leq 0.001$.

Fig. 1

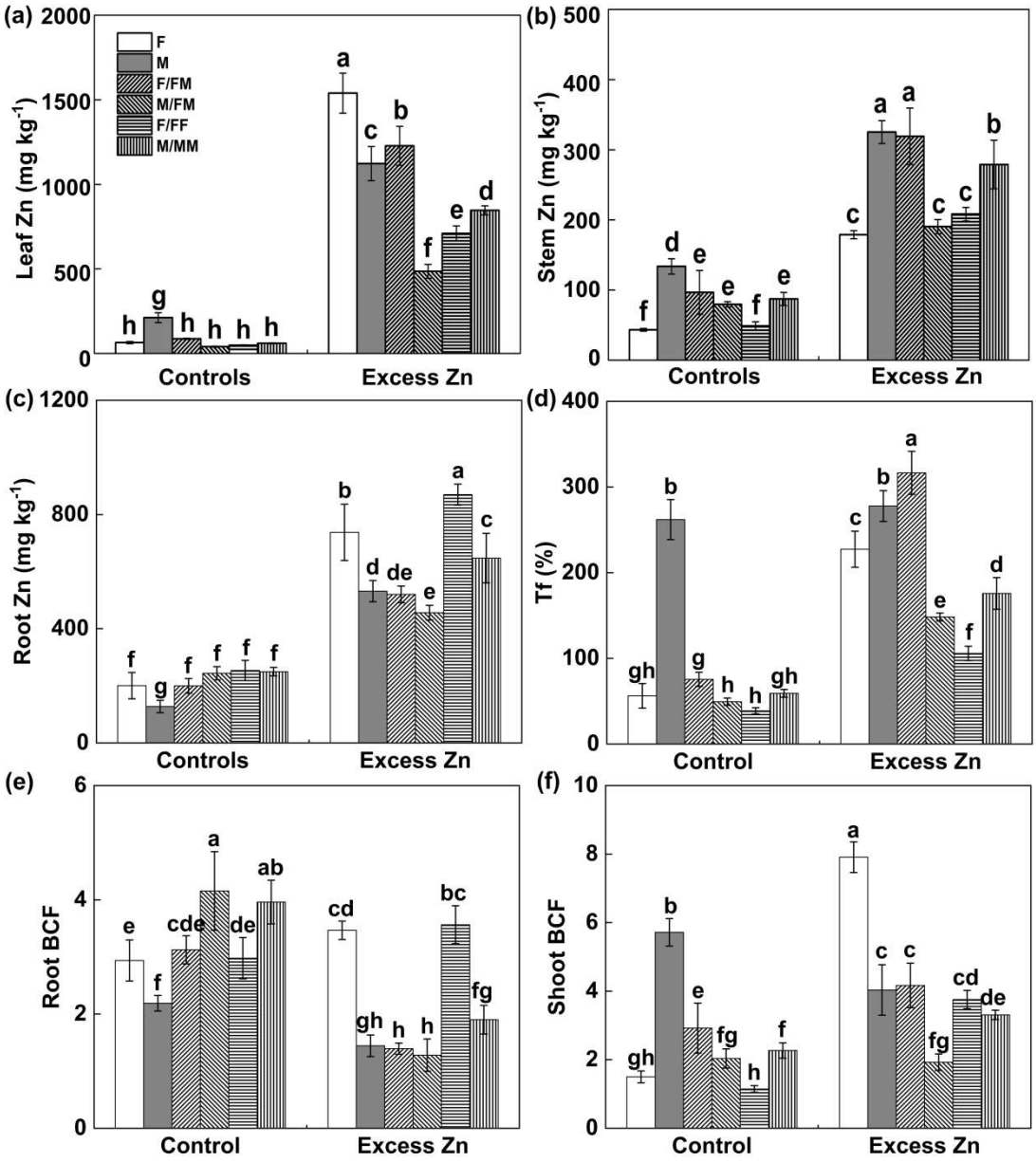

(a)
Leaf Zn (mg kg-1)
F
M
F/FM
M/FM
F/FF
M/MM
Controls
Excess Zn
(b)
Stem Zn (mg kg-1)
Controls
Excess Zn
(c)
Root Zn (mg kg-1)
Controls
Excess Zn
(d)
Tf (%)
Control
Excess Zn
(e)
Root BCF
Control
Excess Zn
(f)
Shoot BCF
Control
Excess Zn

Fig. 2

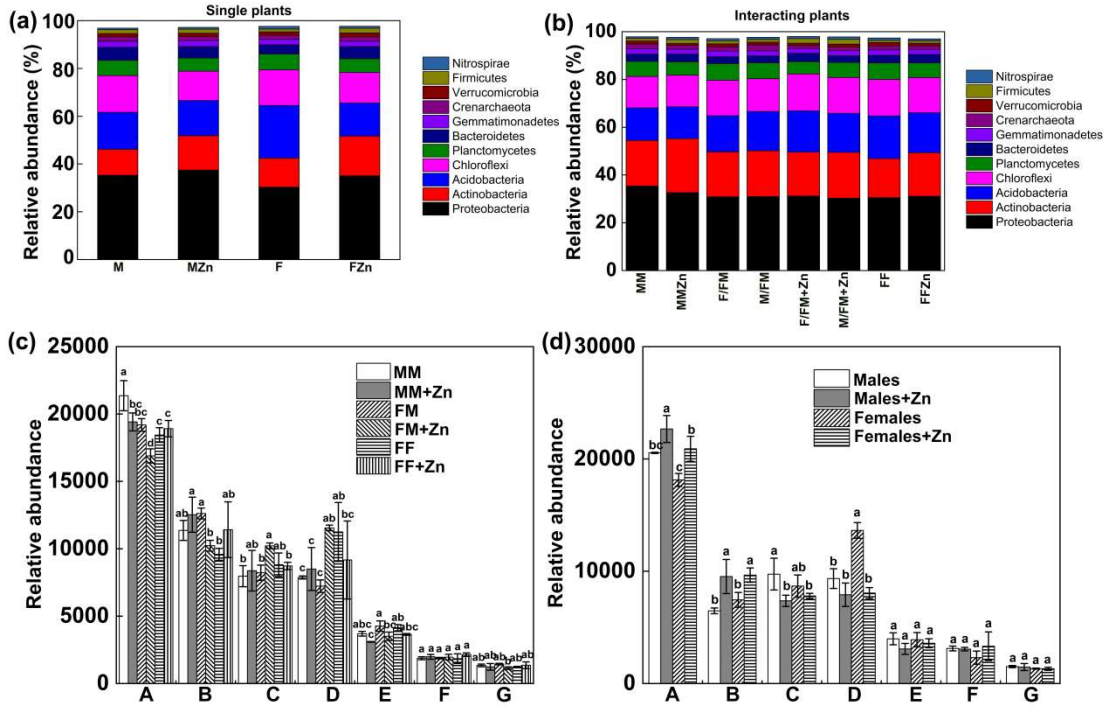

(a)
Single plants
Relative abundance (%)
M
MZn
F
FZn
Nitrospirae
Firmicutes
Verrucomicrobia
Crenarchaeota
Gemmatimonadetes
Bacteroidetes
Planctomycetes
Chloroflexi
Acidobacteria
Actinobacteria
Proteobacteria
(b)
Interacting plants
Relative abundance (%)
MM
MMZn
F/FM
M/FM
F/FM+Zn
M/FM+Zn
FF
FFZn
(c)
Relative abundance
MM
MM+Zn
FM
FM+Zn
FF
FF+Zn
A
B
C
D
E
F
G
(d)
Relative abundance
Males
Males+Zn
Females
Females+Zn

Fig. 3

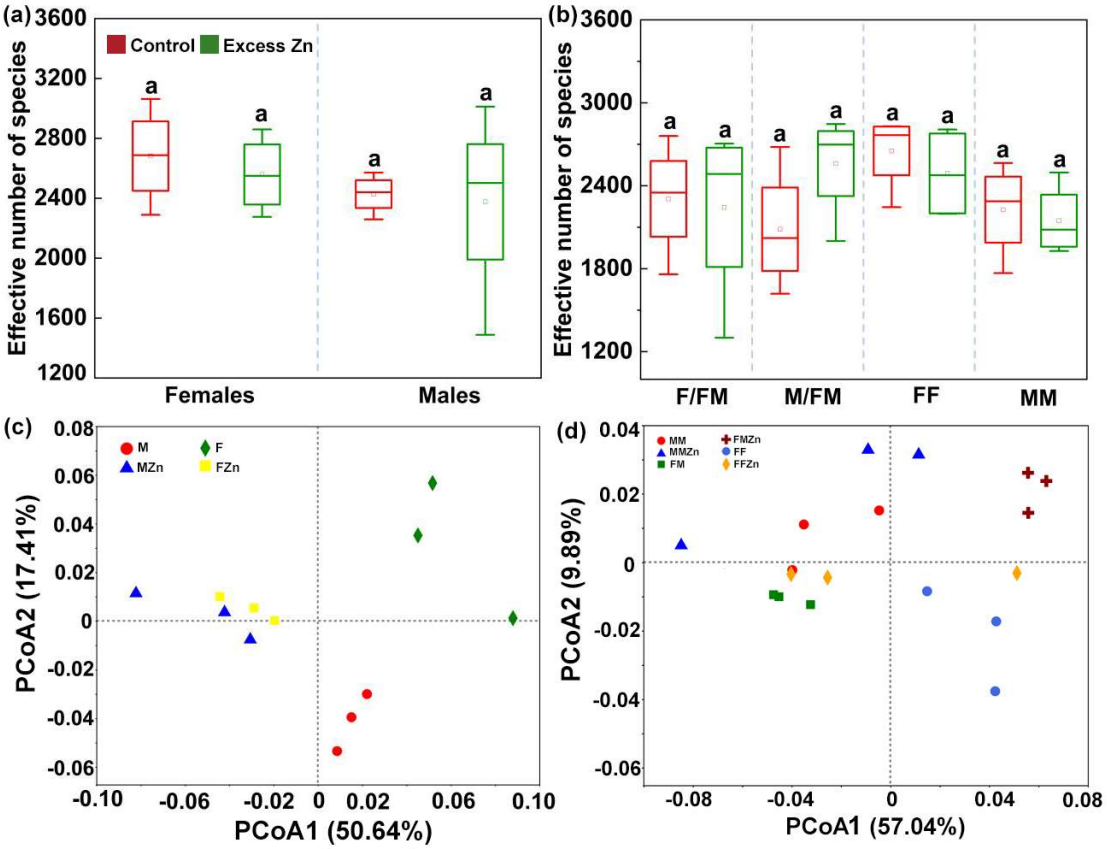

(a)
Control
Excess Zn
Effective number of species
Females
Males
(b)
Effective number of species
F/FM
M/FM
FF
MM
(c)
M
F
MZn
FZn
PCoA2 (17.41%)
PCoA1 (50.64%)
(d)
MM
FMZn
MMZn
FF
FM
FFZn
PCoA2 (9.89%)
PCoA1 (57.04%)

Fig. 4

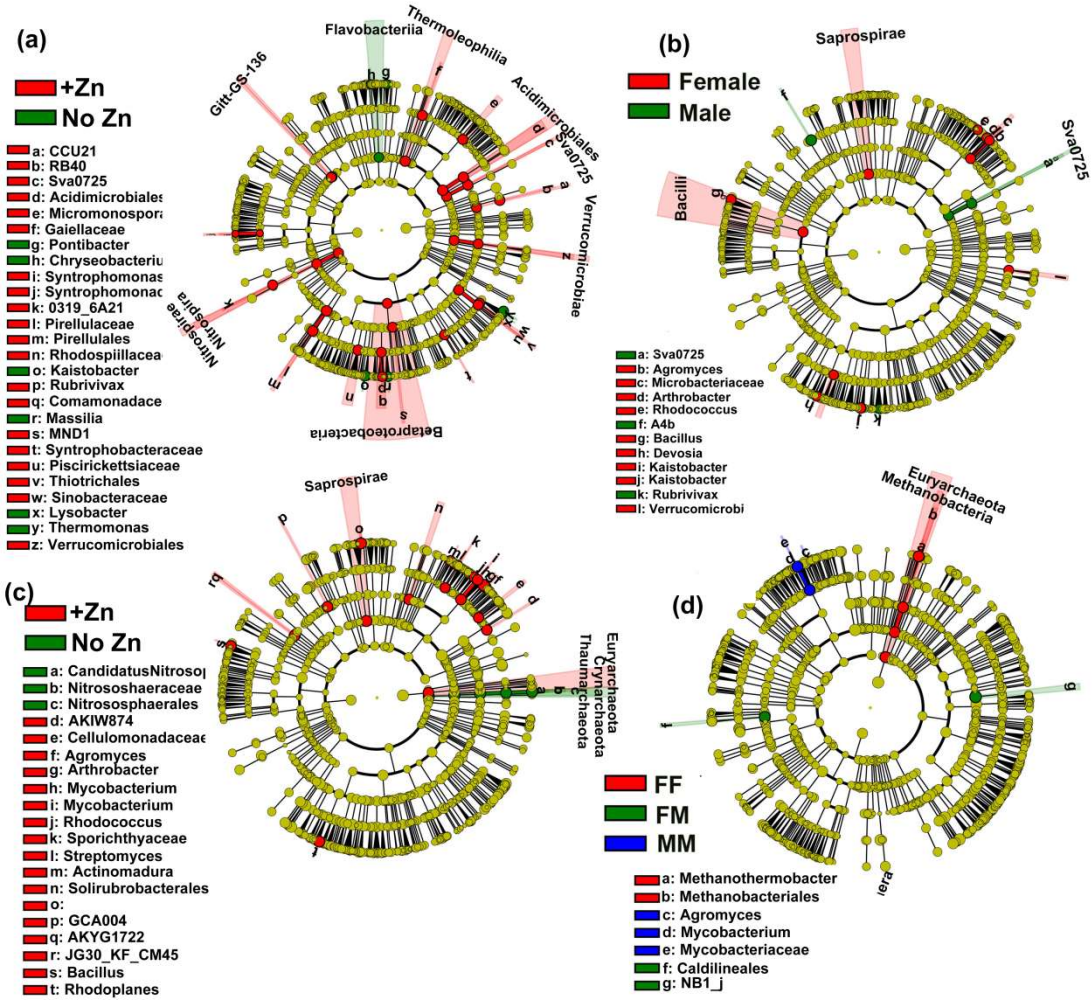

(a)
+Zn
No Zn
a: CCU21
b: RB40
c: Sva0725
d: Acidimicrobiales
e: Micromonospor
f: Gaiellaceae
g: Pontibacter
h: Chryseobacteriu
i: Syntrophomonas
j: Syntrophomonac
k: 0319_6A21
l: Pirellulaceae
m: Pirellulales
n: Rhodospiillacea
o: Kaistobacter
p: Rubrivivax
q: Comamonadace
r: Massilia
s: MND1
t: Syntrophobacteraceae
u: Piscirickettsiaceae
v: Thiotrichales
w: Sinobacteraceae
x: Lysobacter
y: Thermomonas
z: Verrucomicrobiales
Flavobacteriia
Thermoleophilia
Gitt-GS-136
Acidimicrobiales
Sva0725
Verrucomicrobiae
Nitrospirae
Nitrospira
Betaproteobacteria
(b)
Female
Male
Saprospirae
Bacilli
Sva0725
a: Sva0725
b: Agromyces
c: Microbacteriaceae
d: Arthrobacter
e: Rhodococcus
f: A4b
g: Bacillus
h: Devosia
i: Kaistobacter
j: Kaistobacter
k: Rubrivivax
l: Verrucomicrobi
(c)
+Zn
No Zn
a: CandidatusNitroso
b: Nitrososhaeraceae
c: Nitrososphaerales
d: AKIW874
e: Cellulomonadaceae
f: Agromyces
g: Arthrobacter
h: Mycobacterium
i: Mycobacterium
j: Rhodococcus
k: Sporichthyaceae
l: Streptomyces
m: Actinomadura
n: Solirubrobacterales
o:
p: GCA004
q: AKYG1722
r: JG30_KF_CM45
s: Bacillus
t: Rhodoplanes
Saprospirae
Euryarchaeota
Crenarchaeota
Thaumarchaeota
(d)
Euryarchaeota
Methanobacteria
FF
FM
MM
a: Methanothermobacter
b: Methanobacteriales
c: Agromyces
d: Mycobacterium
e: Mycobacteriaceae
f: Caldilineales
g: NB1_j

Fig. 5

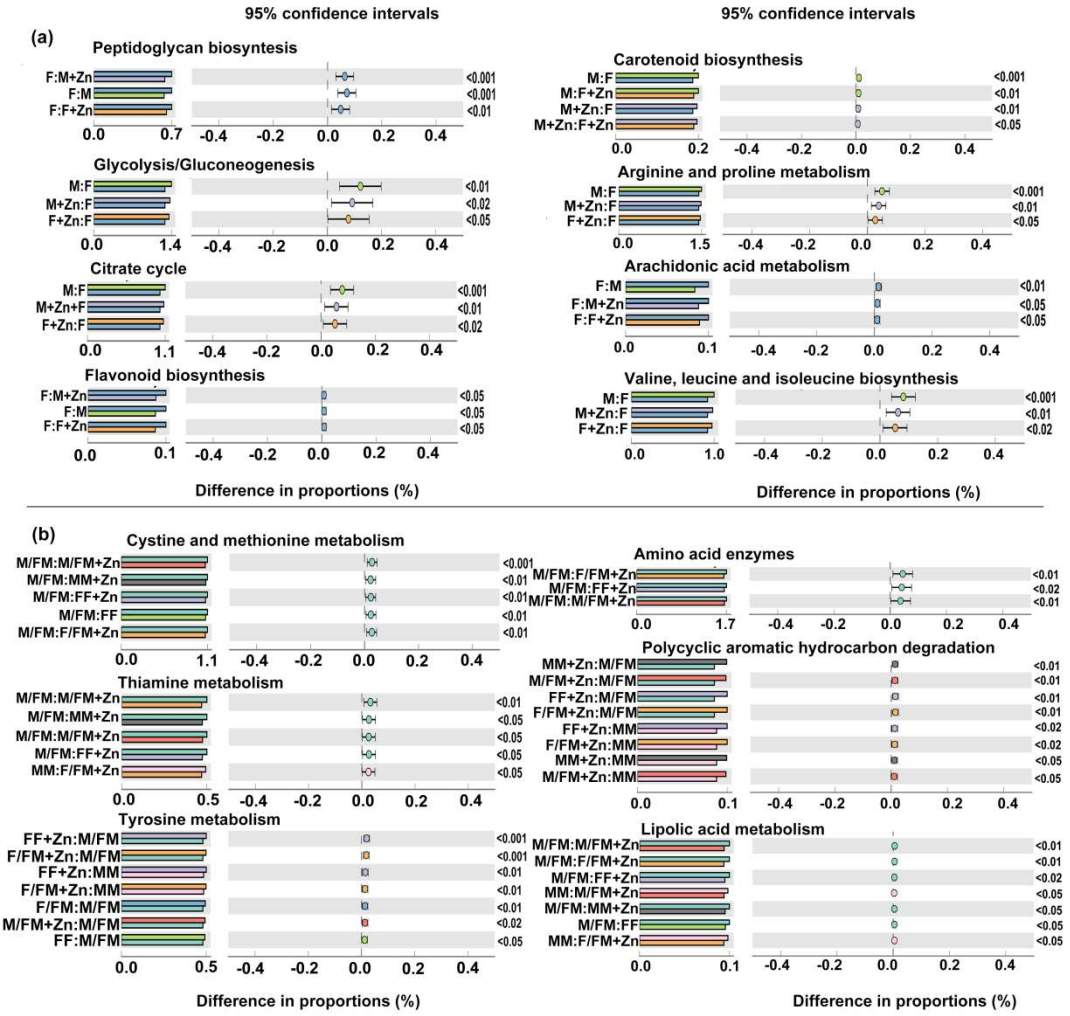
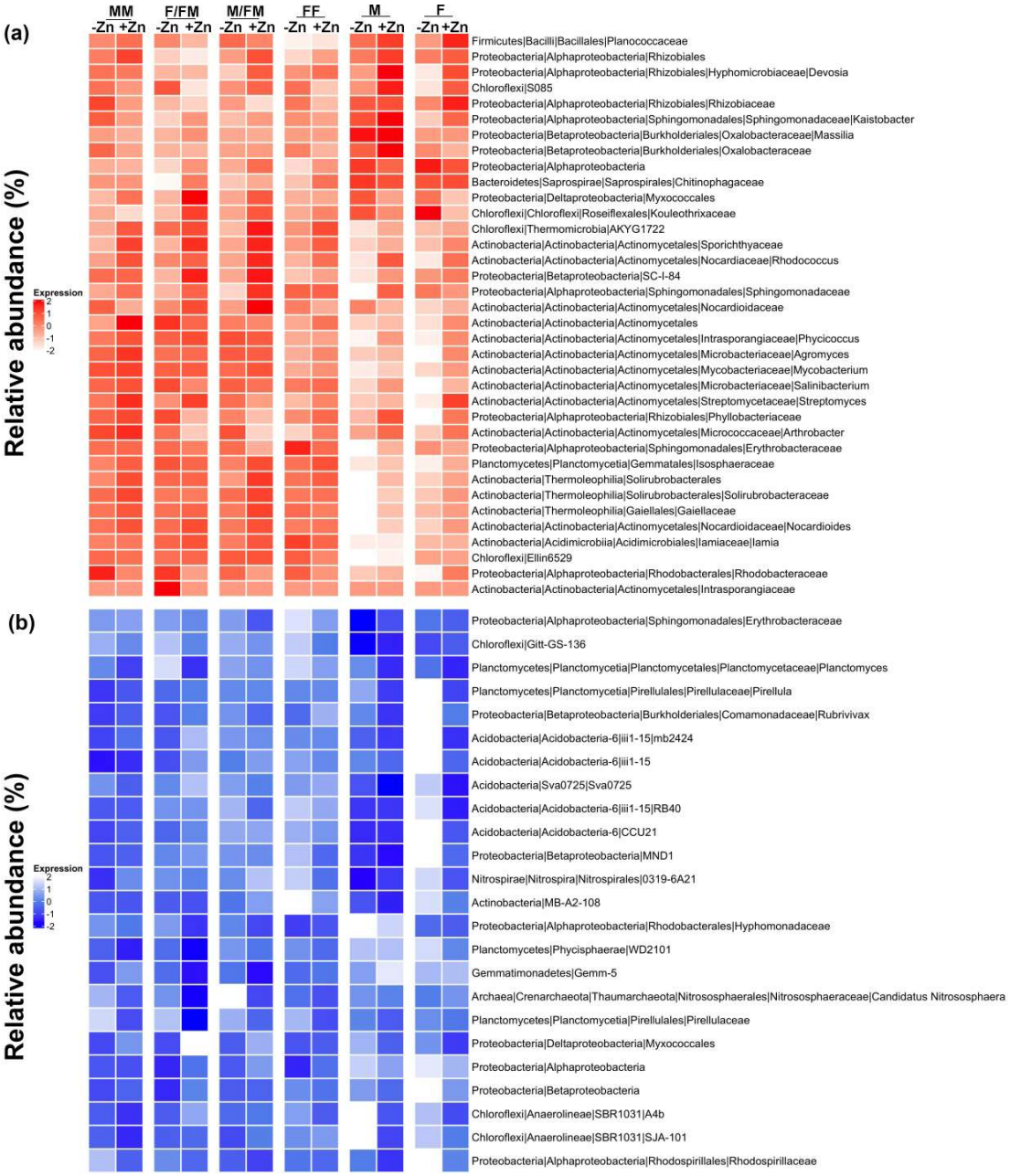

(a)
95% confidence intervals
Peptidoglycan biosynthesis
F:M+Zn
F:M
F:F+Zn
<0.001
<0.001
<0.01
Glycolysis/Gluconeogenesis
M:F
M+Zn:F
F+Zn:F
<0.01
<0.02
<0.05
Citrate cycle
M:F
M+Zn+F
F+Zn:F
<0.001
<0.01
<0.02
Flavonoid biosynthesis
F:M+Zn
F:M
F:F+Zn
<0.05
<0.05
<0.05
Difference in proportions (%)
95% confidence intervals
Carotenoid biosynthesis
M:F
M:F+Zn
M+Zn:F
M+Zn:F+Zn
<0.001
<0.01
<0.01
<0.05
Arginine and proline metabolism
M:F
M+Zn:F
F+Zn:F
<0.001
<0.01
<0.05
Arachidonic acid metabolism
F:M
F:M+Zn
F:F+Zn
<0.01
<0.05
<0.05
Valine, leucine and isoleucine biosynthesis
M:F
M+Zn:F
F+Zn:F
<0.001
<0.01
<0.02
Difference in proportions (%)
(b)
Cystine and methionine metabolism
M/FM:M/FM+Zn
M/FM:MM+Zn
M/FM:FF+Zn
M/FM:FF
M/FM:F/FM+Zn
<0.001
<0.01
<0.01
<0.01
<0.01
Thiamine metabolism
M/FM:M/FM+Zn
M/FM:MM+Zn
M/FM:M/FM+Zn
M/FM:FF+Zn
MM:F/FM+Zn
<0.01
<0.05
<0.05
<0.05
<0.05
Tyrosine metabolism
FF+Zn:M/FM
F/FM+Zn:M/FM
FF+Zn:MM
F/FM+Zn:MM
F/FM:M/FM
M/FM+Zn:M/FM
FF:M/FM
<0.001
<0.001
<0.01
<0.01
<0.01
<0.02
<0.05
Difference in proportions (%)
Amino acid enzymes
M/FM:F/FM+Zn
M/FM:FF+Zn
M/FM:M/FM+Zn
<0.01
<0.02
<0.01
Polycyclic aromatic hydrocarbon degradation
MM+Zn:M/FM
M/FM+Zn:M/FM
FF+Zn:M/FM
F/FM+Zn:M/FM
FF+Zn:MM
F/FM+Zn:MM
MM+Zn:MM
M/FM+Zn:MM
<0.01
<0.01
<0.01
<0.01
<0.02
<0.02
<0.05
<0.05
Lipolic acid metabolism
M/FM:M/FM+Zn
M/FM:F/FM+Zn
M/FM:FF+Zn
MM:M/FM+Zn
M/FM:MM+Zn
M/FM:FF
MM:F/FM+Zn
<0.01
<0.01
<0.02
<0.05
<0.05
<0.05
<0.05
Difference in proportions (%)

Fig. 6


(a)
MM
F/FM
M/FM
FF
M
F
-Zn +Zn
Relative abundance (%)
Expression
2
1
0
-1
-2
Firmicutes|Bacilli|Bacillales|Planococcaceae
Proteobacteria|Alphaproteobacteria|Rhizobiales
Proteobacteria|Alphaproteobacteria|Rhizobiales|Hyphomicrobiaceae|Devosia
Chloroflexi|S085
Proteobacteria|Alphaproteobacteria|Rhizobiales|Rhizobiaceae
Proteobacteria|Alphaproteobacteria|Sphingomonadales|Sphingomonadaceae|Kaistobacter
Proteobacteria|Betaproteobacteria|Burkholderiales|Oxalobacteraceae|Massilia
Proteobacteria|Betaproteobacteria|Burkholderiales|Oxalobacteraceae
Proteobacteria|Alphaproteobacteria
Bacteroidetes|Saprospirae|Saprospirales|Chitinophagaceae
Proteobacteria|Deltaproteobacteria|Myxococcales
Chloroflexi|Chloroflexi|Roseiflexales|Kouleothrixaceae
Chloroflexi|Thermomicrobia|AKYG1722
Actinobacteria|Actinobacteria|Actinomycetales|Sporichthyaceae
Actinobacteria|Actinobacteria|Actinomycetales|Nocardiaceae|Rhodococcus
Proteobacteria|Betaproteobacteria|SC-I-84
Proteobacteria|Alphaproteobacteria|Sphingomonadales|Sphingomonadaceae
Actinobacteria|Actinobacteria|Actinomycetales|Nocardioidaceae
Actinobacteria|Actinobacteria|Actinomycetales
Actinobacteria|Actinobacteria|Actinomycetales|Intrasporangiaceae|Phycicoccus
Actinobacteria|Actinobacteria|Actinomycetales|Microbacteriaceae|Agromyces
Actinobacteria|Actinobacteria|Actinomycetales|Mycobacteriaceae|Mycobacterium
Actinobacteria|Actinobacteria|Actinomycetales|Microbacteriaceae|Salinibacterium
Actinobacteria|Actinobacteria|Actinomycetales|Streptomycetaceae|Streptomyces
Proteobacteria|Alphaproteobacteria|Rhizobiales|Phyllobacteriaceae
Actinobacteria|Actinobacteria|Actinomycetales|Micrococcaceae|Arthrobacter
Proteobacteria|Alphaproteobacteria|Sphingomonadales|Erythrobacteraceae
Planctomycetes|Planctomycetia|Gemmatales|Isosphaeraceae
Actinobacteria|Thermoleophilia|Solirubrobacterales
Actinobacteria|Thermoleophilia|Solirubrobacterales|Solirubrobacteraceae
Actinobacteria|Thermoleophilia|Gaiellales|Gaiellaceae
Actinobacteria|Actinobacteria|Actinomycetales|Nocardioidaceae|Nocardioides
Actinobacteria|Acidimicrobiia|Acidimicrobiales|Iamiaceae|Iamia
Chloroflexi|Ellin6529
Proteobacteria|Alphaproteobacteria|Rhodobacterales|Rhodobacteraceae
Actinobacteria|Actinobacteria|Actinomycetales|Intrasporangiaceae
(b)
Relative abundance (%)
Expression
2
1
0
-1
-2
Proteobacteria|Alphaproteobacteria|Sphingomonadales|Erythrobacteraceae
Chloroflexi|Gitt-GS-136
Planctomycetes|Planctomycetia|Planctomycetales|Planctomycetaceae|Planctomyces
Planctomycetes|Planctomycetia|Pirellulales|Pirellulaceae|Pirellula
Proteobacteria|Betaproteobacteria|Burkholderiales|Comamonadaceae|Rubrivivax
Acidobacteria|Acidobacteria-6|iii1-15|mb2424
Acidobacteria|Acidobacteria-6|iii1-15
Acidobacteria|Sva0725|Sva0725
Acidobacteria|Acidobacteria-6|iii1-15|RB40
Acidobacteria|Acidobacteria-6|CCU21
Proteobacteria|Betaproteobacteria|MND1
Nitrospirae|Nitrospira|Nitrospirales|0319-6A21
Actinobacteria|MB-A2-108
Proteobacteria|Alphaproteobacteria|Rhodobacterales|Hyphomonadaceae
Planctomycetes|Phycisphaerae|WD2101
Gemmatimonadetes|Gemm-5
Archaea|Crenarchaeota|Thaumarchaeota|Nitrososphaerales|Nitrososphaeraceae|Candidatus Nitrososphaera
Planctomycetes|Planctomycetia|Pirellulales|Pirellulaceae
Proteobacteria|Deltaproteobacteria|Myxococcales
Proteobacteria|Alphaproteobacteria
Proteobacteria|Betaproteobacteria
Chloroflexi|Anaerolineae|SBR1031|A4b
Chloroflexi|Anaerolineae|SBR1031|SJA-101
Proteobacteria|Alphaproteobacteria|Rhodospirillales|Rhodospirillaceae